

GENETIC VACCINES DIRECTED AGAINST BACTERIAL EXOTOXINS

STATEMENT REGARDING

FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0001] This invention was made in part with Government support under Grant Number U01HL66952-01 awarded by the National Heart, Lung, and Blood Institute (NHLBI). The Government may have certain rights in this invention.

FIELD OF THE INVENTION

[0002] This invention pertains to vaccines directed against exotoxins of pathogenic bacteria.

BACKGROUND OF THE INVENTION

[0003] In the last 50 years, only 236 people have been infected with anthrax in the United States. Although primarily a veterinary disease, the recent anthrax bioterrorism threat suggests that the incidence of anthrax in humans likely will increase. The disease is initiated by contact with anthrax spores and manifests as inhaled, cutaneous, and gastrointestinal forms, all of which can be fatal. Inhalation anthrax, however, has the highest mortality, with a survival rate of only 60% in the recent U.S. bioterrorism incidents (see, e.g., Ingelsby et al., *JAMA*, 287, 2236-2252 (2002)).

[0004] The pathogenesis of many bacterial infections is dependent on extracellular proteins known as exotoxins. Exotoxins cause pathogenesis by a number of mechanisms, including tissue invasion, cell lysis, effects on neurotransmitter uptake and release, and disruption of cellular homeostasis. With respect to *Bacillus anthracis*, the causative agent of anthrax, pathogenesis is elicited by three exotoxins known as protective antigen (PA), edema factor (EF), and lethal factor (LF). PA is a proteolytically activated heptamer which binds to a specific cellular receptor and facilitates intracellular translocation of EF and/or LF. LF is a metalloprotease which acts on a variety of substrates including mitogen-activated protein kinase. EF induces fluid loss, possibly through elevation of intracellular cyclic AMP (camp) levels. *Bacillus anthracis* exotoxins are binary in that two polypeptides are required for toxicity.

[0005] The anthrax vaccine that is currently available in the United States consists of a cell-free filtrate of a nonencapsulated attenuated strain of *B. anthracis* (Bioport Corporation, Lansing, Michigan), of which protective antigen is the major component (see, e.g., Puziss et al., *J. Bacteriol.*, 85, 230-236 (1962), and Puziss et al., *Appl. Microbiol.*, 11, 330-334 (1963)). The safety and efficacy of this vaccine in humans, however, remains the focus of

intense investigation (see, e.g., Inglesby et al., *supra*). Indeed, several drawbacks associated with the vaccine have been reported, including the need for frequent boosters, the apparent inability to protect adequately against certain strains of *B. anthracis*, and occasional local immunogenicity (see, e.g., Ivins et al., *Eur. J. Epidemiol.*, 4, 12-19 (1988)). In addition, an experimental vaccine based on recombinant PA with an Alhydrogel adjuvant is currently being developed by the U.S. Army, but has not yet been tested in humans (see, e.g., Ivins et al., *Vaccine*, 16, 1141-1148 (1998)).

[0006] Accordingly, there remains a need for alternative compositions and methods for protection against anthrax infection that elicit a rapid and efficient immune response in a broad spectrum of the population. The invention provides such a composition and method. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

[0007] The invention provides a gene transfer vector comprising a nucleic acid sequence which encodes at least an immunogenic portion of one or more exotoxins of *Bacillus anthracis* and a heterologous sorting signal, wherein the nucleic acid sequence comprises codons expressed more frequently in humans than in *Bacillus anthracis*. The invention also provides a method of producing an immune response against *Bacillus anthracis* in a host, which method comprises administering to the host the above-described gene transfer vector, wherein the nucleic acid sequence is expressed to produce the immunogenic portion of the one or more exotoxins in the host, thereby producing an immune response against *Bacillus anthracis*.

DETAILED DESCRIPTION OF THE INVENTION

[0008] The invention provides a gene transfer vector comprising a nucleic acid sequence which encodes at least an immunogenic portion of one or more exotoxins of *Bacillus anthracis* and a heterologous sorting signal. The nucleic acid sequence comprises codons expressed more frequently in humans than in *Bacillus anthracis*. The invention also provides a method of producing an immune response against *Bacillus anthracis* in a host, which method comprises administering to the host the above-described gene transfer vector, wherein the nucleic acid sequence is expressed to produce the immunogenic portion of the one or more exotoxins in the host, thereby producing an immune response against *Bacillus anthracis*. The use of a gene transfer vector, particularly a replication-deficient adenoviral vector, which encodes, using human-preferred codons, an immunogenic portion of one or more *B. anthracis* exotoxins, especially in combination with a heterologous sorting signal, offers an improvement over previously described *B. anthracis* vaccines by optimizing the

humoral immune response directed against the pathogen and minimizing the number of booster administrations required. Various aspects of the inventive gene transfer vector and method are discussed below. Although each parameter is discussed separately, the inventive gene transfer vector and method comprise combinations of the parameters set forth below to evoke protection against anthrax infection in a human. Accordingly, any combination of parameters can be used according to the inventive gene transfer vector and the inventive method.

Gene Transfer Vector

[0009] A “gene transfer vector” is any molecule or composition that has the ability to carry a heterologous nucleic acid sequence into a suitable host cell where synthesis of the encoded protein takes place. Typically and preferably, a gene transfer vector is a nucleic acid molecule that has been engineered, using recombinant DNA techniques that are known in the art, to incorporate the heterologous nucleic acid sequence. Desirably, the gene transfer vector is comprised of DNA. Examples of suitable DNA-based gene transfer vectors include plasmids and viral vectors. However, gene transfer vectors that are not based on nucleic acids, such as liposomes, are also known and used in the art. The inventive gene transfer vector can be based on a single type of nucleic acid (e.g., a plasmid) or non-nucleic acid molecule (e.g., a lipid or a polymer). Alternatively, the gene transfer vector can be a combination of a nucleic acid molecule and a non-nucleic acid molecule (i.e., “chimeric”). For example, a plasmid harboring the heterologous nucleic acid sequence can be formulated with a lipid or a polymer as a delivery vehicle. Such a gene transfer vector is referred to herein as a “plasmid-lipid complex” or a “plasmid-polymer” complex, respectively. The inventive gene transfer vector can be integrated into the host cell genome, or can be present in the host cell in the form of an episome.

[0010] Preferably, the gene transfer vector is a viral vector. Suitable viral vectors include, for example, retroviral vectors, herpes simplex virus (HSV)-based vectors, parvovirus-based vectors, e.g., adeno-associated virus (AAV)-based vectors, AAV-adenoviral chimeric vectors, and adenovirus-based vectors. These viral vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *Molecular Cloning, a Laboratory Manual*, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[0011] Retrovirus is an RNA virus capable of infecting a wide variety of host cells. Upon infection, the retroviral genome integrates into the genome of its host cell and is replicated along with host cell DNA, thereby constantly producing viral RNA and any nucleic acid sequence incorporated into the retroviral genome. As such, long-term

expression of a therapeutic factor(s) is achievable when using retrovirus. Retroviruses contemplated for use in gene therapy are relatively non-pathogenic, although pathogenic retroviruses exist. When employing pathogenic retroviruses, e.g., human immunodeficiency virus (HIV) or human T-cell lymphotropic viruses (HTLV), care must be taken in altering the viral genome to eliminate toxicity to the host. A retroviral vector additionally can be manipulated to render the virus replication-deficient. As such, retroviral vectors are considered particularly useful for stable gene transfer *in vivo*. Lentiviral vectors, such as HIV-based vectors, are exemplary of retroviral vectors used for gene delivery. Unlike other retroviruses, HIV-based vectors are known to incorporate their passenger genes into non-dividing cells and, therefore, can be of use in treating persistent forms of disease.

[0012] An HSV-based viral vector is suitable for use as a gene transfer vector to introduce a nucleic acid into numerous cell types. The mature HSV virion consists of an enveloped icosahedral capsid with a viral genome consisting of a linear double-stranded DNA molecule that is 152 kb. Most replication-deficient HSV vectors contain a deletion to remove one or more intermediate-early genes to prevent replication. Advantages of the HSV vector are its ability to enter a latent stage that can result in long-term DNA expression and its large viral DNA genome that can accommodate exogenous DNA inserts of up to 25 kb. Of course, the ability of HSV to promote long-term production of exogenous protein is potentially disadvantageous in terms of short-term treatment regimens. However, one of ordinary skill in the art has the requisite understanding to determine the appropriate vector for a particular situation. HSV-based vectors are described in, for example, U.S. Patents 5,837,532, 5,846,782, 5,849,572, and 5,804,413, and International Patent Applications WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583.

[0013] AAV vectors are viral vectors of particular interest for use in gene therapy protocols. AAV is a DNA virus, which is not known to cause human disease. The AAV genome is comprised of two genes, *rep* and *cap*, flanked by inverted terminal repeats (ITRs), which contain recognition signals for DNA replication and packaging of the virus. AAV requires co-infection with a helper virus (i.e., an adenovirus or a herpes simplex virus), or expression of helper genes, for efficient replication. AAV can be propagated in a wide array of host cells including human, simian, and rodent cells, depending on the helper virus employed. An AAV vector used for administration of a nucleic acid sequence typically has approximately 96% of the parental genome deleted, such that only the ITRs remain. This eliminates immunologic or toxic side effects due to expression of viral genes. If desired, the AAV *rep* protein can be co-administered with the AAV vector to enable integration of the AAV vector into the host cell genome. Host cells comprising an integrated AAV genome show no change in cell growth or morphology (see, e.g., U.S.

Patent 4,797,368). As such, prolonged expression of therapeutic factors from AAV vectors can be useful in treating persistent and chronic diseases.

[0014] The viral vector is most preferably an adenoviral vector. Adenovirus (Ad) is a 36 kb double-stranded DNA virus that efficiently transfers DNA *in vivo* to a variety of different target cell types. The adenoviral vector can be produced in high titers and can efficiently transfer DNA to replicating and non-replicating cells. The adenoviral vector genome can be generated using any species, strain, subtype, mixture of species, strains, or subtypes, or chimeric adenovirus as the source of vector DNA. Adenoviral stocks that can be employed as a source of adenovirus can be amplified from the human adenoviral serotypes 1 through 51, which are currently available from the American Type Culture Collection (ATCC, Manassas, VA), or from any other serotype of human adenovirus available from any other source. For instance, an adenovirus can be of subgroup A (e.g., serotypes 12, 18, and 31), subgroup B (e.g., serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (e.g., serotypes 1, 2, 5, and 6), subgroup D (e.g., serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, and 42-48), subgroup E (e.g., serotype 4), subgroup F (e.g., serotypes 40 and 41), an unclassified serogroup (e.g., serotypes 49 and 51), or any other adenoviral serotype. Given that the human adenovirus serotype 5 (Ad5) genome has been completely sequenced, the adenoviral vector of the invention is described herein with respect to the Ad5 serotype.

[0015] In addition to human adenovirus, the adenoviral vector can be generated using a non-human primate adenovirus, in which case, the adenovirus is preferably a chimpanzee adenovirus. Conceivably, the administration of a chimpanzee adenoviral vector to a human host may avoid the immune response often elicited by human adenoviral vectors as a result of pre-existing immunity to human adenovirus (see, e.g., U.S. Patent 6,083,716). In this regard, the major neutralizing epitope of certain strains of chimpanzee adenovirus differ from those of corresponding human strains (see, e.g., Farina et al., *J. Virol.*, 75, 11603-11613 (2001)). Thus, humans have no pre-existing immunity against such strains of chimpanzee adenovirus. Adenoviral stocks that can be employed as a source of chimpanzee adenovirus can be amplified from the chimpanzee adenoviral strains C1 or CV68 ("C68"), which are currently available from the American Type Culture Collection (ATCC, Manassas, VA), or from any other strain of chimpanzee adenovirus available from any other source. The C1 strain is most similar to human adenovirus of subgroup B, while the C68 strain is most similar to serotype 4 of human adenovirus (subgroup E). Accordingly, the gene regions of strain C1 have been identified by analyzing the sequence homology to the known gene regions of human Ad3, Ad5, and Ad7. Similarly, the gene regions of strain C68 have been identified through sequence comparisons with human Ad4 and Ad5 (see U.S. Patent 6,083,716). Strain C68 has been shown to bind the same

coxsackievirus and adenovirus receptor (“CAR”) as human Ad5 for cell entry (see, e.g., Farina et al., *supra*).

[0016] Adenoviral vectors are well known in the art and are described in, for example, U.S. Patents 5,559,099, 5,712,136, 5,731,190, 5,837,511, 5,846,782, 5,851,806, 5,962,311, 5,965,541, 5,981,225, 5,994,106, 6,020,191, 6,083,716, 6,113,913, and 6,482,616, U.S. Patent Application Publication Nos. 2001/0043922 A1, 2002/0004040 A1, 2002/0031831 A1, and 2002/0110545 A1, International Patent Applications WO 95/34671, WO 97/21826, and WO 00/00628, and Thomas Shenk, “Adenoviridae and their Replication,” and M. S. Horwitz, “Adenoviruses,” Chapters 67 and 68, respectively, in *Virology*, B. N. Fields et al., eds., 3d ed., Raven Press, Ltd., New York (1996).

[0017] Preferably, the adenoviral vector is replication-deficient. By “replication-deficient” is meant that the adenoviral vector comprises a genome that lacks at least one replication-essential gene function. A deficiency in a gene, gene function, gene, or genomic region, as used herein, is defined as a deletion of sufficient genetic material of the viral genome to impair or obliterate the function of the gene whose nucleic acid sequence was deleted in whole or in part. Replication-essential gene functions are those gene functions that are required for replication (i.e., propagation) of a replication-deficient adenoviral vector. Replication-essential gene functions are encoded by, for example, the adenoviral early regions (e.g., the E1, E2, and E4 regions), late regions (e.g., the L1-L5 regions), genes involved in viral packaging (e.g., the IVa2 gene), and virus-associated RNAs (e.g., VA-RNA I and/or VA-RNA II). Preferably, the replication-deficient adenoviral vector comprises an adenoviral genome deficient in at least one replication-essential gene function of one or more regions of an adenoviral genome (e.g., two or more regions of an adenoviral genome so as to result in a multiply replication-deficient adenoviral vector). The one or more regions of the adenoviral genome are preferably selected from the group consisting of the E1, E2, and E4 regions. More preferably, the replication-deficient adenoviral vector comprises a deficiency in at least one replication-essential gene function of the E1 region (denoted an E1-deficient adenoviral vector), particularly a deficiency in a replication-essential gene function of each of the adenoviral E1A region and the adenoviral E1B region. In addition to such a deficiency in the E1 region, the recombinant adenovirus also can have a mutation in the major late promoter (MLP), as discussed in International Patent Application WO 00/00628. More preferably, the vector is deficient in at least one replication-essential gene function of the E1 region and at least part of the nonessential E3 region (e.g., an Xba I deletion of the E3 region) (denoted an E1/E3-deficient adenoviral vector). As with human adenovirus, chimpanzee adenovirus strain C68 can be rendered replication-deficient by deletion of the E1A and E1B gene regions.

[0018] Preferably, the adenoviral vector is “multiply deficient,” meaning that the adenoviral vector is deficient in one or more gene functions required for viral replication in each of two or more regions of the adenoviral genome. For example, the aforementioned E1-deficient or E1/E3-deficient adenoviral vector can be further deficient in at least one replication-essential gene function of the E4 region (denoted an E1/E4-deficient adenoviral vector). An adenoviral vector deleted of the entire E4 region can elicit a lower host immune response.

[0019] Alternatively, the adenoviral vector lacks replication-essential gene functions in all or part of the E1 region and all or part of the E2 region (denoted an E1/E2-deficient adenoviral vector). Adenoviral vectors lacking replication-essential gene functions in all or part of the E1 region, all or part of the E2 region, and all or part of the E3 region also are contemplated herein. If the adenoviral vector of the invention is deficient in a replication-essential gene function of the E2A region, the vector preferably does not comprise a complete deletion of the E2A region, which is less than about 230 base pairs in length. Generally, the E2A region of the adenovirus codes for a DBP (DNA binding protein), a polypeptide required for DNA replication. DBP is composed of 473 to 529 amino acids depending on the viral serotype. It is believed that DBP is an asymmetric protein that exists as a prolate ellipsoid consisting of a globular Ct with an extended Nt domain. Studies indicate that the Ct domain is responsible for DBP’s ability to bind to nucleic acids, bind to zinc, and function in DNA synthesis at the level of DNA chain elongation. However, the Nt domain is believed to function in late gene expression at both transcriptional and post-transcriptional levels, is responsible for efficient nuclear localization of the protein, and also may be involved in enhancement of its own expression. Deletions in the Nt domain between amino acids 2 to 38 have indicated that this region is important for DBP function (Brough et al., *Virology*, 196, 269-281 (1993)). While deletions in the E2A region coding for the Ct region of the DBP have no effect on viral replication, deletions in the E2A region which code for amino acids 2 to 38 of the Nt domain of the DBP impair viral replication. It is preferable that the multiply replication-deficient adenoviral vector contain this portion of the E2A region of the adenoviral genome. In particular, for example, the desired portion of the E2A region to be retained is that portion of the E2A region of the adenoviral genome which is defined by the 5' end of the E2A region, specifically positions Ad5(23816) to Ad5(24032) of the E2A region of the adenoviral genome of serotype Ad5.

[0020] The adenoviral vector can be deficient in replication-essential gene functions of only the early regions of the adenoviral genome, only the late regions of the adenoviral genome, and both the early and late regions of the adenoviral genome. The adenoviral vector also can have essentially the entire adenoviral genome removed, in which case it is preferred that at least either the viral inverted terminal repeats (ITRs) and one or more

promoters or the viral ITRs and a packaging signal are left intact (i.e., an adenoviral amplicon). The larger the region of the adenoviral genome that is removed, the larger the piece of exogenous nucleic acid sequence that can be inserted into the genome. For example, given that the adenoviral genome is 36 kb, by leaving the viral ITRs and one or more promoters intact, the exogenous insert capacity of the adenovirus is approximately 35 kb. Alternatively, a multiply deficient adenoviral vector that contains only an ITR and a packaging signal effectively allows insertion of an exogenous nucleic acid sequence of approximately 37-38 kb. Of course, the inclusion of a spacer element in any or all of the deficient adenoviral regions will decrease the capacity of the adenoviral vector for large inserts. Suitable replication-deficient adenoviral vectors, including multiply deficient adenoviral vectors, are disclosed in U.S. Patents 5,851,806 5,994,106, and 6,482,616, and International Patent Applications WO 95/34671 and WO 97/21826.

[0021] It should be appreciated that the deletion of different regions of the adenoviral vector can alter the immune response of the mammal. In particular, the deletion of different regions can reduce the inflammatory response generated by the adenoviral vector. Furthermore, the adenoviral vector's coat protein can be modified so as to decrease the adenoviral vector's ability or inability to be recognized by a neutralizing antibody directed against the wild-type coat protein, as described in International Patent Application WO 98/40509.

[0022] The adenoviral vector, when multiply replication-deficient, especially in replication-essential gene functions of the E1 and E4 regions, preferably includes a spacer element to provide viral growth in a complementing cell line similar to that achieved by singly replication-deficient adenoviral vectors, particularly an adenoviral vector comprising a deficiency in the E1 region. The spacer element can contain any sequence or sequences which are of the desired length. The spacer element sequence can be coding or non-coding and native or non-native with respect to the adenoviral genome, but does not restore the replication-essential function to the deficient region. In the absence of a spacer, production of fiber protein and/or viral growth of the multiply replication-deficient adenoviral vector is reduced by comparison to that of a singly replication-deficient adenoviral vector. However, inclusion of the spacer in at least one of the deficient adenoviral regions, preferably the E4 region, can counteract this decrease in fiber protein production and viral growth. The use of a spacer in an adenoviral vector is described in U.S. Patent 5,851,806.

[0023] Alternatively, the adenoviral vector can be a conditionally-replicating adenoviral vector, which is engineered to replicate under conditions pre-determined by the practitioner. For example, replication-essential gene functions, e.g., gene functions encoded by the adenoviral early regions, can be operably linked to an inducible, repressible, or tissue-specific transcription control sequence, e.g., promoter. In this embodiment, replication

requires the presence or absence of specific factors that interact with the transcription control sequence. Replication of the adenoviral vector can be limited to a target tissue, thereby allowing greater distribution of the vector throughout the tissue while exploiting adenovirus' natural ability to lyse cells during the replication cycle, thereby providing a mechanism of destroying infected cells. Conditionally-replicating adenoviral vectors are described further in U.S. Patent 5,998,205.

[0024] Construction of adenoviral vectors is well understood in the art. Adenoviral vectors can be constructed and/or purified using the methods set forth, for example, in U.S. Patents 5,965,358, 5,994,128, 6,033,908, 6,168,941, 6,329,200, 6,383,795, 6,440,728, 6,447,995, and 6,475,757, and International Patent Applications WO 98/53087, WO 98/56937, WO 99/15686, WO 99/54441, WO 00/12765, WO 01/77304, and WO 02/29388, as well as other references identified herein. Moreover, numerous adenoviral vectors are available commercially. The production of adenoviral gene transfer vectors involves using standard molecular biological techniques such as those described in, for example, Sambrook et al., *supra*, Watson et al., *supra*, Ausubel et al., *supra*, and several of the other references mentioned herein.

[0025] Replication-deficient adenoviral vectors are typically produced in complementing cell lines that provide gene functions not present in the replication-deficient adenoviral vectors, but required for viral propagation, at appropriate levels in order to generate high titers of viral vector stock. A preferred cell line complements for at least one, and preferably all, replication-essential gene functions not present in a replication-deficient adenovirus. The complementing cell line can complement for a deficiency in at least one replication-essential gene function encoded by the early regions, late regions, viral packaging regions, virus-associated RNA regions, or combinations thereof, including all adenoviral functions (e.g., to enable propagation of adenoviral amplicons, which comprise minimal adenoviral sequences, such as only inverted terminal repeats (ITRs) and the packaging signal or only ITRs and an adenoviral promoter). Most preferably, the complementing cell line complements for a deficiency in at least one replication-essential gene function (e.g., two or more replication-essential gene functions) of the E1 region of the adenoviral genome, particularly a deficiency in a replication-essential gene function of each of the E1A and E1B regions. In addition, the complementing cell line can complement for a deficiency in at least one replication-essential gene function of the E2 (particularly as concerns the adenoviral DNA polymerase and terminal protein) and/or E4 regions of the adenoviral genome. Desirably, a cell that complements for a deficiency in the E4 region comprises the E4-ORF6 gene sequence and produces the E4-ORF6 protein. Such a cell desirably comprises at least ORF6 and no other ORF of the E4 region of the adenoviral genome.

[0026] The cell line preferably is further characterized in that it contains the complementing genes in a non-overlapping fashion with the adenoviral vector, which minimizes, and practically eliminates, the possibility of the vector genome recombining with the cellular DNA. Accordingly, the presence of replication-competent adenoviruses (RCA) is minimized if not avoided in the vector stock, which, therefore, is suitable for certain therapeutic purposes, especially gene therapy purposes. The lack of RCA in the vector stock avoids the replication of the adenoviral vector in non-complementing cells.

[0027] The construction of complementing cell lines involves standard molecular biology and cell culture techniques, such as those described by Sambrook et al., *supra*, and Ausubel et al., *supra*. Complementing cell lines for producing the gene transfer vector (e.g., human or chimpanzee adenoviral vector) include, but are not limited to, 293 cells (described in, e.g., Graham et al., *J. Gen. Virol.*, 36, 59-72 (1977)), PER.C6 cells (described in, e.g., International Patent Application WO 97/00326, and U.S. Patents 5,994,128 and 6,033,908), and 293-ORF6 cells (described in, e.g., International Patent Application WO 95/34671 and Brough et al., *J. Virol.*, 71, 9206-9213 (1997)).

Nucleic Acid Sequence Encoding B. anthracis Exotoxin

[0028] The inventive gene transfer vector comprises a nucleic acid sequence which encodes at least an immunogenic portion of one or more exotoxins of *Bacillus anthracis* (i.e., one or more nucleic acid sequences encoding one or more immunogenic portions of one or more exotoxins) and a heterologous sorting signal. The gene transfer vector comprises at least one nucleic acid sequence as described herein, i.e., the gene transfer vector can comprise one nucleic acid sequence as described herein or more than one nucleic acid sequence as described herein (i.e., two or more nucleic acid sequences). The nucleic acid sequence encoding the immunogenic portion can be obtained from any source, e.g., isolated from nature, synthetically generated, isolated from a genetically engineered organism, and the like. An ordinarily skilled artisan will appreciate that any type of nucleic acid sequence (e.g., DNA, RNA, and cDNA) that can be inserted into a gene transfer vector can be used in connection with the invention. The nucleic acid sequence can be recombinantly produced, or can be a genomic nucleic acid sequence. Typically and preferably, the nucleic acid sequence is transcribed and translated into a peptide, polypeptide, or protein. In some cases, however, the nucleic acid sequence is not translated, such as when the nucleic acid sequence encodes an antisense molecule or a ribozyme.

[0029] When the gene transfer vector is a replication-deficient adenovirus, the nucleic acid sequence encoding the protein is preferably located in the E1 region of the adenoviral genome. The insertion of a nucleic acid sequence into the adenoviral genome (e.g., the E1

region of the adenoviral genome) can be facilitated by known methods, for example, by the introduction of a unique restriction site at a given position of the adenoviral genome.

[0030] Whatever type of nucleic acid sequence is used, the nucleic acid sequence preferably encodes an immunogenic portion of one or more exotoxins of *Bacillus anthracis*. By “immunogenic portion” is meant any peptide, polypeptide, or portion thereof, that elicits an immune response (e.g., humoral and/or cell-mediated) against the organism from which it is obtained from, derived from, or based upon when introduced into a host. A sequence is “obtained” from a source when it is isolated from that source. A sequence is “derived” from a source when it is isolated from a source but modified in any suitable manner (e.g., by deletion, substitution, insertion, or other modification to the sequence) so as not to disrupt the normal function of the source gene. A sequence is “based upon” a source when the sequence is a sequence more than about 70% homologous (preferably more than about 80% homologous, more preferably more than about 90% homologous, and most preferably more than about 95% homologous) to the source but obtained through synthetic procedures (e.g., polynucleotide synthesis, directed evolution, etc.). Determining the degree of homology can be accomplished using any suitable method (e.g., BLASTnr, provided by GenBank).

[0031] Assessing the immunogenicity of an exotoxin, or portion thereof, can be determined using routine immunology techniques that are known in the art. With respect to humoral (i.e., antibody, immunoglobulin, or B cell) immune responses, for example, a suitable laboratory animal (e.g., a rabbit or mouse) can be immunized with a nucleic acid sequence encoding a candidate immunogenic portion of a *B. anthracis* exotoxin. Serum levels of antibodies specific for the polypeptide, protein, or protein portion encoded by the nucleic acid sequence can be detected and measured using any suitable method, including radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) (see, e.g., Abbas et al., eds., *Cellular and Molecular Immunology*, 4th ed., W.B. Saunders Company, Philadelphia (2000)). The cell-mediated (i.e., T cell) immune response elicited by expression of a nucleic acid sequence encoding a candidate immunogenic *B. anthracis* exotoxin can be assessed using, for example, the enzyme-linked immunospot (ELISPOT) assay. The ELISPOT assay enables detection of cells stimulated (e.g., by antigen) to produce cytokines. Preferably, the source cells for ELISPOT are isolated from the spleen and/or lymph nodes of immunized animals. The absence of a humoral or cell-mediated response to the polypeptide, protein, or protein portion encoded by the nucleic acid sequence indicates that the nucleic acid sequence does not encode an immunogenic portion of a *B. anthracis* exotoxin. These methods, however, are merely exemplary. Indeed, any method for determining the immunogenicity of a candidate exotoxin or portion thereof is within the scope of the invention.

[0032] In a preferred embodiment of the invention, the nucleic acid sequence encodes at least an immunogenic portion of one or more exotoxins of *Bacillus anthracis*. The one or more exotoxins preferably are selected from protective antigen (PA), lethal factor (LF), and edema factor (EF), taken individually or in any combination. As is common with other exotoxins that act intracellularly, the *B. anthracis* exotoxins are binary in that at least two different exotoxins are required to induce pathogenicity. In this respect, the nucleic acid sequence can encode at least an immunogenic portion of one exotoxin of *B. anthracis*, or, alternatively, an immunogenic portion of each of two or more exotoxins of *B. anthracis*. Preferably, the nucleic acid sequence encodes at least an immunogenic portion of protective antigen; however, nucleic acid sequences encoding immunogenic portions of lethal factor and/or edema factor are also within the scope of the invention.

[0033] Protective antigen is a 735 amino acid protein organized into four domains, and binds to an unidentified receptor on the surface of mammalian cells. When bound to its receptor, PA is cleaved by furin or a furin-like protease, which releases an amino-terminal PA fragment (PA₂₀) and leads to heptamerization of the remainder of the protein (PA₆₃) on the cell surface. PA₆₃ forms a complex with EF and LF, which is internalized by the cell via endocytosis. Acidification of the endosome induces a conformational change in the PA₆₃ heptamer, converting PA₆₃ into a membrane-spanning pore. EF and LF are then translocated through the pore into the cytosolic environment. PA appears to have no further role in the intoxication process (see, e.g., Mogridge et al., *J. Bact.*, 183, 2111-2116 (2001), and Sellman et al., *J. Biol. Chem.*, 276, 8371-8376 (2001)).

[0034] In one embodiment of the invention, the nucleic acid sequence preferably encodes a wild-type immunogenic portion of a *B. anthracis* exotoxin. Alternatively, however, when expression of a wild-type *B. anthracis* exotoxin is pathogenic in a host, such as when an immunogenic portion of two *B. anthracis* exotoxins (e.g., PA and LF, or PA and EF) is expressed in a host, the nucleic acid encodes a mutant form of the exotoxin that is immunogenic, but not pathogenic, in a host. A mutant exotoxin is preferably produced by introducing one or more mutations (e.g., point mutations, deletions, insertions, etc.) into the nucleic acid sequence encoding a naturally occurring exotoxin. Such mutations are introduced in the nucleic acid sequence to effect one or more amino acid substitutions in an encoded exotoxin. Thus, where mutations are introduced in the nucleic acid sequence encoding the exotoxin, such mutations desirably will effect a substitution in the encoded exotoxin whereby codons encoding positively-charged residues (H, K, and R) are substituted with codons encoding positively-charged residues, codons encoding negatively-charged residues (D and E) are substituted with codons encoding negatively-charged residues, codons encoding neutral polar residues (C, G, N, Q, S, T, and Y) are substituted with codons encoding neutral polar residues, and codons encoding neutral

non-polar residues (A, F, I, L, M, P, V, and W) are substituted with codons encoding neutral non-polar residues. In addition, the nucleic acid sequence can encode a homolog of an immunogenic portion of a wild-type or mutant *B. anthracis* exotoxin. A homolog of an immunogenic portion of a *B. anthracis* exotoxin, whether wild-type or mutant, can be any peptide, polypeptide, or portion thereof, that is more than about 70% identical (preferably more than about 80% identical, more preferably more than about 90% identical, and most preferably more than about 95% identical) to the immunogenic portion of the exotoxin at the amino acid level. The degree of amino acid identity can be determined using any method known in the art, such as the BLAST sequence database. Furthermore, a homolog of the exotoxin can be any peptide, polypeptide, or portion thereof, which hybridizes to the exotoxin under at least moderate, preferably high, stringency conditions. Exemplary moderate stringency conditions include overnight incubation at 37° C in a solution comprising 20% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1x SSC at about 37-50° C, or substantially similar conditions, e.g., the moderately stringent conditions described in Sambrook et al., *supra*. High stringency conditions are conditions that use, for example (1) low ionic strength and high temperature for washing, such as 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate (SDS) at 50° C, (2) employ a denaturing agent during hybridization, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin (BSA)/0.1% Ficoll/0.1% polyvinylpyrrolidone (PVP)/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride and 75 mM sodium citrate at 42° C, or (3) employ 50% formamide, 5x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42° C, with washes at (i) 42° C in 0.2x SSC, (ii) 55° C in 50% formamide, and (iii) 55° C in 0.1x SSC (preferably in combination with EDTA). Additional details and an explanation of stringency of hybridization reactions are provided in, e.g., Ausubel et al., *supra*.

[0035] When the nucleic acid sequence encodes a mutant form of, for example, protective antigen, the nucleic acid sequence can encode an oligomerization mutant of PA. It is believed that certain mutations in domain three of PA are required for heptamerization of PA. For example, a single mutation of PA amino acid 512 has been shown to be sufficient to prevent oligomerization (see, e.g., Mogridge et al., *supra*). However, any mutation that inhibits oligomerization of PA while preserving immunogenicity is within the scope of the invention. PA oligomerization mutants are described in, for example, Mogridge et al., *supra*. In addition or alternatively, the nucleic acid sequence can encode a

PA mutant that is unable to translocate EF and LF across the endosome membrane into the cytosol. Such PA translocation mutants preferably have amino acid substitutions at residues 397, 425, and/or 427 of the PA polypeptide. PA translocation mutants are described in, for example, Sellman et al., *supra*, and Sellman et al., *Science*, 292, 695-697 (2001).

[0036] It is believed that the immune response to one antigen encoded by a pathogenic organism (e.g., a bacterium or virus) can be enhanced by co-expression of another unrelated antigen (see, e.g., Price et al., *Infect. Immun.*, 69, 4509-4515 (2001)). Thus, in one embodiment of the invention, the nucleic acid sequence can encode an immunogenic portion of each of two or more exotoxins of *B. anthracis*. Preferably, the nucleic acid sequence encodes an immunogenic portion of PA and an immunogenic portion of LF or EF. As discussed above, PA, LF, and EF are non-toxic individually, but when expressed in binary or tertiary combinations, they produce toxic shock-like symptoms and, in many cases, death. In this embodiment, the nucleic acid sequence preferably encodes a mutant immunogenic portion of the *B. anthracis* PA exotoxin. In this respect, the immunogenic portion of PA can be derived from a nucleic acid sequence encoding any mutant or variant PA that retains immunogenicity, but not pathogenicity, in a host, such as those mutants or variants described herein. The immunogenic portion of LF or EF can be the wild-type, full length LF or EF protein, the PA-binding domain of LF or EF (i.e., amino acids 10-254) (see, e.g., Lacy et al., *J. Biol. Chem.*, 277, 3006-3010 (2002)), or any other immunogenic portion of the LF or EF proteins. The nucleic acid can encode the immunogenic portion of each of two or more *B. anthracis* exotoxins in any combination. That is, the nucleic acid sequence can encode a mutant form of PA together with a wild-type or truncated form of LF and/or EF.

[0037] The expression of the nucleic acid sequence in the inventive gene transfer vector is controlled by a suitable expression control sequence operably linked to the nucleic acid sequence. An "expression control sequence" is any nucleic acid sequence that promotes, enhances, or controls expression (typically and preferably transcription) of another nucleic acid sequence. Suitable expression control sequences include constitutive promoters, inducible promoters, repressible promoters, and enhancers. The nucleic acid sequence can be regulated by its endogenous promoter or by a nonnative promoter sequence. Examples of suitable nonnative promoters include the cytomegalovirus (CMV) immediate early (IE) promoter, the phosphoglycerate kinase (PGK) promoter, the long terminal repeat promoter of the Rous sarcoma virus (LTR-RSV), the sheep metallothionein promoter, and the human ubiquitin C promoter. Alternatively, expression of the nucleic acid sequence can be controlled by a chimeric promoter sequence. The promoter sequence is "chimeric" when it comprises at least two nucleic acid sequence portions obtained from, derived from, or based upon at least two different sources (e.g., two different regions of an organism's genome,

two different organisms, or an organism combined with a synthetic sequence). The expression of the nucleic acid sequence in the inventive gene transfer vector is preferably regulated by the CMV IE promoter. Other preferred expression control sequences include the chicken β -actin promoter, the LTR-RSV promoter, the dendritic cell-specific dectin 2 promoter, and a chimeric expression control sequence comprising the CMV IE enhancer region and the chicken β -actin promoter. Suitable expression control sequences can be determined using eukaryotic expression systems such as are generally described in Sambrook et al., *supra*, and by using reporter gene systems (see, e.g., Taira et al., *Gene*, 263, 285-292 (2001)).

[0038] Preferably, the nucleic acid sequence in the inventive gene transfer vector further comprises a transcription-terminating region such as a polyadenylation sequence located 3' of the nucleic acid sequence. Any suitable polyadenylation sequence can be used, including a synthetic polyadenylation sequence, as well as the polyadenylation sequence of BGH (Bovine Growth Hormone), polyoma virus, SV40 (Human Sarcoma Virus-40), TK (Thymidine Kinase), EBV (Epstein Barr Virus), and the papillomaviruses, including human papillomaviruses and BPV (Bovine Papilloma Virus).

Heterologous Sorting Signal

[0039] The nucleic acid sequence contained in the inventive gene transfer vector encodes a heterologous sorting signal. The term "sorting signal," as used herein, refers to an amino acid sequence found in a protein that selectively guides the distribution of the protein to specific subcellular compartments. For example, sorting signals can direct proteins to intracellular organelles for uptake and processing. The nucleic acid sequence in the inventive gene transfer can further comprise a heterologous signal peptide. The term "signal peptide," as used herein, refers to a peptide, typically located at the amino terminus of a protein, which targets the protein to specific cellular compartments, such as the endoplasmic reticulum, and directs secretion of the mature protein from the cell in which it is produced. Unlike sorting signals, which can comprise a portion of the mature protein in which they are found, signal peptides typically are removed from a precursor polypeptide and, thus, are not present in mature proteins. The sorting signal and signal peptide are "heterologous" in that either is not obtained from, derived from, or based upon a naturally occurring sorting signal or signal peptide of *B. anthracis*. By "naturally occurring" is meant that the sorting signal or signal peptide is encoded by a nucleic acid sequence that can be found in nature and has not been synthetically modified. Notwithstanding the foregoing, however, the nucleic acid sequence that encodes a heterologous sorting signal or signal peptide can be naturally found in *B. anthracis*, but located at a nonnative position with

respect to the immunogenic portion of one or more exotoxins of *B. anthracis* and/or operably linked to a nonnative promoter.

[0040] Preferably, the heterologous sorting signal directs the exotoxin to a subcellular sorting pathway. The subcellular sorting pathway is selected from the group consisting of an extracellular pathway, a cytoplasmic pathway, a cell membrane pathway, a lysosome pathway, an endoplasmic reticulum pathway, and a degradative pathway, although sorting signals which direct the exotoxin to other subcellular sorting pathways known in the art are within the scope of the invention.

[0041] The heterologous sorting signal desirably directs the immunogenic portion of the inventive *B. anthracis* exotoxin to a subcellular sorting pathway that is involved in antigen presentation. It is believed that protection against anthrax infection primarily depends upon humoral immune responses (see, e.g., Gu et al., *Vaccine*, 17, 340-344 (1999); Pitt et al., *J. Appl. Microbiol.*, 87, 304 (1999); Welkos et al., *Microbiology*, 147, 1677-1685 (2001)). The humoral immune response is mediated, in part, by the major histocompatibility class II (MHC II) antigen presentation pathway. Antigens can be presented on MHC II peptides through an exogenous mechanism, involving antigen uptake by antigen presenting cells from the extracellular environment and subsequent degradation in acidic endosomal and lysosomal vesicles, leading to epitope presentation by MHC II peptides to CD4+ T cells ("helper T cells") at the cell surface. Alternatively, antigens produced within antigen presenting cells also can be presented via the MHC II pathway (see, e.g., U.S. Patent 6,500,641). This mechanism for MHC II presentation, while not completely understood, has been demonstrated by targeting viral antigens to the lysosomal compartment by addition of a sorting signal isolated from various lysosomal-associated proteins.

[0042] Desirably, the heterologous sorting signal is any suitable heterologous sorting signal that directs the immunogenic portion of the one or more exotoxins of *Bacillus anthracis* to a lysosomal compartment in a host cell such that it is presented by MHC II peptides at the cell surface, thereby eliciting a humoral immune response against *B. anthracis*. Most preferably, the heterologous sorting signal is a lysosomal-associated membrane protein-1 (LAMP-1) sorting signal. LAMP-1 is a type 1 transmembrane protein that localizes predominantly to lysosomes and late endosomes (see, e.g., Wu et al., *Proc. Natl. Acad. Sci., USA*, 92, 11671-11675 (1995)). The LAMP-1 sorting signal comprises the transmembrane and cytoplasmic domains of the LAMP-1 protein. The nucleic acid sequence in the inventive gene transfer vector, therefore, preferably encodes the transmembrane and cytoplasmic domains of LAMP-1. Another suitable heterologous sorting signal includes, for example, the C-terminal tail of the lysosomal integral membrane protein II (LIMP-II). One of ordinary skill in the art will appreciate that the presence of a heterologous signal peptide in the polypeptide or protein encoded by the inventive nucleic

acid sequence mediates translocation of the polypeptide or protein to the endoplasmic reticulum, from which the polypeptide or protein is directed to lysosomal or endosomal compartments via the heterologous sorting signal. Any signal peptide that directs secretion of the polypeptide or protein encoded by the nucleic acid sequence is suitable for use in the inventive gene transfer vector. The heterologous signal peptide is preferably a LAMP-1 signal peptide. The nucleic acid sequence in the inventive gene transfer vector desirably is constructed such that, when expressed, the heterologous signal peptide and the heterologous sorting signal are located at the N-terminus and C-terminus, respectively, of the polypeptide or protein encoded by the nucleic acid sequence. In addition to signal peptides, other mechanisms for secretion may be employed, such as, for example, truncation, deletion, or point mutation of secretion-inhibiting sequences present in the nucleic acid sequence.

Humanized B. anthracis Exotoxin Sequences

[0043] In accordance with the invention, the nucleic acid sequence which encodes at least an immunogenic portion of one or more exotoxins of *Bacillus anthracis* comprises codons expressed more frequently in humans than in *Bacillus anthracis*. While the genetic code is generally universal across species, the choice among synonymous codons is often species-dependent. Infrequent usage of a particular codon by an organism likely reflects a low level of the corresponding transfer RNA (tRNA) in the organism. Thus, introduction of a nucleic acid sequence into an organism which comprises codons that are not frequently utilized in the organism may result in limited expression of the nucleic acid sequence. One of ordinary skill in the art would appreciate that, to achieve maximum protection against *B. anthracis* infection, the inventive gene transfer vector must be capable of expressing high levels of *B. anthracis* exotoxins in a human host. In this respect, the inventive nucleic acid sequence encodes the native amino acid sequence of the immunogenic portion of the one or more *B. anthracis* exotoxins, but comprises codons that are expressed more frequently in humans than in *Bacillus anthracis*. Such modified nucleic acid sequences are commonly described in the art as “humanized” or as utilizing “human-preferred” codons.

[0044] In general, *B. anthracis* toxin genes are rich in adenosine (A) and thymine (T) nucleotides, resulting in a preference for codons that use an A or T at the third position, some of which are not commonly used in human genes. Thus, in the context of the invention, a *B. anthracis* nucleic acid sequence is said to be “humanized” if at least about 60% (e.g., at least about 70%, at least about 80%, or at least about 90%) of the wild-type codons in the nucleic acid sequence are modified to encode human-preferred codons. That is, a *B. anthracis* nucleic acid sequence is humanized if at least about 60% of the codons encoded therein are human-preferred codons. Preferred humanized nucleic acid sequences encoding PA, LF, and EF are set forth in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3,

respectively. However, the invention is not limited to these exemplary sequences. Indeed, genetic sequences can vary between different strains, and this natural scope of allelic variation is included within the scope of the invention. Additionally and alternatively, the humanized nucleic acid sequence encoding *B. anthracis* PA, LF, and/or EF exotoxins can be any sequence that hybridizes to SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3 under at least moderate, preferably high, stringency conditions, such as those described herein. Determining the degree of homology can be accomplished using any suitable method (e.g., BLASTnr, provided by GenBank).

Gene Transfer Vector Targeting

[0045] To enhance the immune response produced by the inventive gene transfer vector when administered to a host, the gene transfer vector desirably is modified such that it preferentially transduces antigen presenting cells. Antigen presenting cells (APCs) include, for example, dendritic cells (DC), macrophages, and monocytes. By transducing and transferring nucleic acid sequences into antigen presenting cells directly, particularly dendritic cells, antigen presentation is enhanced, conceivably resulting in lower required doses of the gene transfer vector as compared to an unmodified gene transfer vector. To direct the inventive gene transfer vector to the desired antigen presenting cells, its natural tropism for other types of cells preferably is attenuated or eliminated.

[0046] In one embodiment of the invention, the gene transfer vector is an adenoviral vector, preferably a replication-deficient adenoviral vector. The coat protein of the adenoviral vector can be manipulated to alter the binding specificity or recognition of the adenoviral vector for a viral receptor on a potential host cell. Such manipulations can include deletion of regions of the fiber, penton, hexon, pIIIa, pVI, and/or pIX, insertions of various native or non-native ligands into portions of the coat protein, and the like. Manipulation of the coat protein can broaden the range of cells infected by the adenoviral vector or enable targeting of the adenoviral vector to a specific cell type.

[0047] For example, in one embodiment, the adenoviral vector comprises a chimeric coat protein (e.g., a fiber, hexon, pIX, pIIIa, or penton protein), which differs from the wild-type (i.e., native) coat protein by the introduction of a nonnative amino acid sequence, preferably at or near the carboxyl terminus. Preferably, the nonnative amino acid sequence is inserted into or in place of an internal coat protein sequence. The nonnative amino acid sequence can be inserted within the internal coat protein sequence or at the end of the internal coat protein sequence. The resultant chimeric viral coat protein is able to direct entry into cells of the adenoviral vector comprising the coat protein that is more efficient than entry into cells of an adenoviral vector that is identical except for comprising a wild-type viral coat protein rather than the chimeric viral coat protein. Preferably, the chimeric

coat protein binds a novel endogenous binding site present on the cell surface that is not recognized, or is poorly recognized, by an adenoviral vector comprising a wild-type coat protein. One direct result of this increased efficiency of entry is that the adenoviral vector can bind to and enter cell types which an adenovirus comprising wild-type coat protein typically cannot enter or can enter with only a low efficiency. If desired, native binding of the adenoviral coat proteins, e.g., the fiber or penton base, can be ablated.

[0048] Adenovirus infection of dendritic cells (DC) is mediated primarily through the interaction of the Arg-Gly-Asp (RGD) domain of the penton base protein and integrin molecules expressed at high levels on the surface of DC. A serotype 5 adenovirus engineered to express an additional RGD domain in the fiber knob infects dendritic cells at least 50-fold more effectively than an adenovirus with a wild-type capsid. Thus, in one embodiment of the invention, the gene transfer vector is an adenoviral vector in which the fiber protein has been manipulated to contain an RGD domain, such that the adenoviral vector transduces antigen presenting cells, preferably dendritic cells, more efficiently than an adenoviral vector with a wild-type fiber protein.

[0049] In another embodiment, the inventive gene transfer vector is an adenoviral vector in which the native tropism of the adenoviral fiber and penton proteins has been ablated. Ablation of native binding of fiber and penton proteins is preferably achieved by deletion of the CAR binding domain and deletion of the RGD domain, respectively (i.e., F⁻/PB⁻). Adenovirus containing deletions of the fiber CAR domain and penton RGD domain recognize and infect antigen presenting cells in the liver more efficiently than an adenovirus comprising wild-type coat proteins. The above-described F⁻/PB⁻ adenoviral vectors can be further manipulated to broaden and enhance targeting to antigen presenting cells. In this embodiment, the fiber protein of an F⁻/PB⁻ adenoviral vector can be engineered to express a ligand that specifically binds, for example, dendritic cells. Such ligands preferably comprise peptide sequences that bind preferentially to dendritic cells versus other cell types, and include, for example, antibodies or fragments thereof that bind specific DC cell surface markers (e.g., CD40). DC-binding peptide sequences can be identified using any suitable technique known in the art, such as phage display libraries. Phage display libraries have been utilized to identify peptide ligands *in vivo* (see, for example, U.S. Patent 5,622,699), and are further described in Nicklin et al., *Mol. Ther.*, 4, 534-542 (2001), and Work et al., *Methods Enzymol.*, 346, 157-176 (2002). Once putative DC-specific ligands are identified, preferential infection and gene transfer to DC can be established *in vitro* prior to assessment of DC tropism *in vivo*, using viral entry assays that employ adenoviruses engineered to express a reporter gene.

[0050] In another embodiment, the gene transfer vector is an adenoviral vector comprising a chimeric virus coat protein not selective for a specific type of eukaryotic cell.

The chimeric coat protein differs from the wild-type coat protein by an insertion of a nonnative amino acid sequence into or in place of an internal coat protein sequence. For example, a ligand comprising about five to about nine lysine residues (preferably seven lysine residues) is attached to the C-terminus of the adenoviral fiber protein via a non-coding spacer sequence. In this embodiment, the chimeric virus coat protein efficiently binds to a broader range of eukaryotic cells than a wild-type virus coat, including dendritic cells, such as described in International Patent Application WO 97/20051.

[0051] The specificity of binding of an adenoviral vector to a given cell also can be adjusted by use of an adenovirus comprising a short-shafted adenoviral fiber gene, as discussed in U.S. Patent 5,962,311. Use of an adenovirus comprising a short-shafted adenoviral fiber gene reduces the level or efficiency of adenoviral fiber binding to its cell-surface receptor and increases adenoviral penton base binding to its cell-surface receptor, thereby increasing the specificity of binding of the adenoviral vector to a given cell. Alternatively, use of an adenovirus comprising a short-shafted fiber enables targeting of the adenovirus to a desired cell-surface receptor by the introduction of a nonnative amino acid sequence either into the penton base or the fiber knob.

[0052] Of course, the ability of an adenoviral vector to recognize a potential host cell can be modulated without genetic manipulation of the coat protein, i.e., through use of a bi-specific molecule. For instance, complexing an adenovirus with a bispecific molecule comprising a penton base-binding domain and a domain that selectively binds a particular cell surface binding site enables the targeting of the adenoviral vector to a particular cell type.

[0053] Suitable modifications to an adenoviral vector are described in U.S. Patents 5,543,328, 5,559,099, 5,712,136, 5,731,190, 5,756,086, 5,770,442, 5,846,782, 5,871,727, 5,885,808, 5,922,315, 5,962,311, 5,965,541, 6,057,155, 6,127,525, 6,153,435, 6,329,190, 6,455,314, and 6,465,253, U.S. Patent Application Publication Nos. 2001/0047081 A1, 2002/0099024 A1, and 2002/0151027 A1, and International Patent Applications WO 96/07734, WO 96/26281, WO 97/20051, WO 98/07865, WO 98/07877, WO 98/40509, WO 98/54346, WO 00/15823, WO 01/58940, and WO 01/92549.

Pharmaceutical Composition

[0054] The pharmaceutical composition of the invention comprises a pharmaceutically acceptable carrier and the inventive gene transfer vector comprising the nucleic acid sequence encoding an immunogenic portion of one or more exotoxins of *Bacillus anthracis*. Any suitable pharmaceutically acceptable carrier can be used within the context of the invention, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the pharmaceutical composition is to be

administered and the particular method used to administer the pharmaceutical composition. The following formulations are merely exemplary and are in no way limiting. However, oral, injectable, and aerosol formulations are preferred.

[0055] Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or orange juice, (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solids or granules, (c) suspensions in an appropriate liquid, and (d) suitable emulsions. Tablet forms can include one or more of lactose, mannitol, corn starch, potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible excipients. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia, emulsions, gels, and the like containing, in addition to the active ingredient, such excipients as are known in the art.

[0056] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[0057] Formulations suitable for aerosol administration comprise the inventive gene transfer vector, alone or in combination with other suitable components, which can be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They also can be formulated as pharmaceuticals for non-pressured preparations, such as in a nebulizer or an atomizer.

[0058] Other suitable formulations are possible, for example, suppositories can be prepared by use of a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration can be presented as pessaries, tampons, creams, gels, pastes, foams, or spray formulas containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

[0059] More preferably, the pharmaceutical composition is formulated to protect the gene transfer vector from damage prior to administration. For example, in embodiments where the gene transfer vector is an adenoviral vector, the particular formulation desirably decreases the light sensitivity and/or temperature sensitivity of the adenoviral vector. Indeed, the pharmaceutical composition will be maintained for various periods of time and, therefore, should be formulated to ensure stability and maximal activity at the time of administration. Typically, the pharmaceutical composition is maintained at a temperature above 0° C, preferably at 4° C or higher (e.g., 4-10° C). In some embodiments, it is desirable to maintain the pharmaceutical composition at a temperature of 10° C or higher (e.g., 10-20° C), 20° C or higher (e.g., 20-25° C), or even 30° C or higher (e.g., 30-40° C). The pharmaceutical composition can be maintained at the aforementioned temperature(s) for at least 1 day (e.g., 7 days (1 week) or more), though typically the time period will be longer, such as at least 3, 4, 5, or 6 weeks, or even longer, such as at least 10, 11, or 12 weeks, prior to administration to a patient. During that time period, the adenoviral gene transfer vector optimally loses no, or substantially no, activity, although some loss of activity is acceptable, especially with relatively higher storage temperatures and/or relatively longer storage times. Preferably, the activity of the adenoviral vector composition decreases about 20% or less, preferably about 10% or less, and more preferably about 5% or less, after any of the aforementioned time periods.

[0060] To this end, the pharmaceutical composition preferably comprises a pharmaceutically acceptable liquid carrier, such as, for example, those described above, and a stabilizing agent selected from the group consisting of polysorbate 80, L-arginine, polyvinylpyrrolidone, α -D-glucopyranosyl α -D-glucopyranoside dihydrate (commonly known as trehalose), and combinations thereof. More preferably, the stabilizing agent is trehalose, or trehalose in combination with polysorbate 80. The stabilizing agent can be present in any suitable concentration in the pharmaceutical composition. When the stabilizing agent is trehalose, the trehalose desirably is present in a concentration of about 2-10% (wt./vol.), preferably about 4-6% (wt./vol.) of the pharmaceutical composition. When trehalose and polysorbate 80 are present in the pharmaceutical composition, the trehalose preferably is present in a concentration of about 4-6% (wt./vol.), more preferably about 5% (wt./vol.), while the polysorbate 80 desirably is present in a concentration of about 0.001-0.01% (wt./vol.), more preferably about 0.0025% (wt./vol.). When a stabilizing agent, e.g., trehalose, is included in the pharmaceutical composition, the pharmaceutically acceptable liquid carrier preferably contains a saccharide other than trehalose. Suitable formulations of the pharmaceutical composition are further described in U.S. Patent 6,225,289 and International Patent Application WO 00/34444.

[0061] When the inventive gene transfer vector is an adenoviral vector, the pharmaceutical composition can further be formulated to reduce adherence loss of the adenoviral vector on devices used to prepare, store, or administer the adenoviral vector, such as glassware, syringes, or needles. Use of such a pharmaceutical composition will extend the shelf life of the pharmaceutical composition, facilitate administration, and increase the efficacy of the inventive method. In this regard, the pharmaceutical composition also can be formulated to enhance the spread of the adenoviral vector throughout the target tissue and/or enhance transduction efficiency. To this end, the pharmaceutical composition also can comprise hyaluronidase, which has been shown to enhance uptake of adenoviral vectors. Addition of proteases to the pharmaceutical composition can enhance the spread of the adenoviral vector throughout the target tissue. The adenoviral vectors of the pharmaceutical composition can be bound to biocompatible solid carriers, such as particulate carriers (e.g., beads, wafers, etc.), that remain in the target tissue due to size, or incorporated into a matrix, such as gel or foam.

[0062] In addition, the pharmaceutical composition can comprise additional therapeutic or biologically-active agents. For example, therapeutic factors useful in the treatment of a particular indication can be present. Factors that control inflammation, such as ibuprofen or steroids, can be part of the pharmaceutical composition to reduce swelling and inflammation associated with *in vivo* administration of the gene transfer vector and physiological distress. Immune system suppressors can be administered with the pharmaceutical composition to reduce any immune response to the gene transfer vector itself or associated with a disorder. Alternatively, immune enhancers can be included in the pharmaceutical composition to upregulate the body's natural defenses against disease. Moreover, cytokines can be administered with the pharmaceutical composition to attract immune effector cells to the infection site.

[0063] Anti-angiogenic factors, such as soluble growth factor receptors, growth factor antagonists, i.e., angiotensin, and the like, also can be part of the pharmaceutical composition. Similarly, vitamins and minerals, anti-oxidants, and micronutrients can be co-administered with the pharmaceutical composition. Antibiotics, i.e., microbicides and fungicides, can be present to reduce the risk of infection associated with gene transfer procedures and other disorders.

Method of Producing an Immune Response

[0064] The invention further provides a method of producing an immune response against *Bacillus anthracis* in a host. The inventive method comprises administering to the host a gene transfer vector having a nucleic acid sequence which encodes at least an immunogenic portion of one or more exotoxins of *Bacillus anthracis* and a heterologous

signal peptide, wherein the nucleic acid sequence comprises codons expressed more frequently in humans than in *Bacillus anthracis*, and wherein the nucleic acid sequence is expressed to produce the immunogenic portion of the one or more exotoxins in the host, thereby producing an immune response against *Bacillus anthracis*. The inventive method desirably produces maximum levels of humoral immunity against *B. anthracis* infection within a short time period, while minimizing or eliminating the need for repeat administrations to maintain immunity. Descriptions of the gene transfer vector, the nucleic acid sequence, the pharmaceutical composition, and components thereof set forth above in connection with the inventive gene transfer vector also are applicable to those same aspects of the aforesaid inventive method.

[0065] The inventive method is desirably performed *in vivo*, preferably within a mammal, and most preferably within a human. When the method is applied *in vivo*, the invention provides a method of administering (i.e., inoculating or immunizing) the inventive gene transfer to a host, most preferably a human host. In accordance with the method, the gene transfer vector, such as is set forth above, is introduced into the host under conditions sufficient for the host to mount an immune response against the immunogenic portion of the one or more exotoxins of *B. anthracis*. While many methods of administration are known in the art, oral administration, intramuscular injection, and subdermal (i.e., subcutaneous) injection are preferred.

[0066] Whatever method is chosen to administer the inventive gene transfer vector in accordance with the inventive method, preferably the gene transfer vector is administered to antigen presenting cells of the host. Most preferably, the gene transfer vector is administered to dendritic cells of the host. As described above in connection with the inventive gene transfer vector, the gene transfer vector can be manipulated to ablate the natural tropism of the gene transfer vector, and introduce a new tropism for antigen presenting cells. The methods for modifying gene transfer vectors, particularly the coat proteins of adenoviral vectors, to preferentially bind dendritic cells are described above, and are applicable to this embodiment of the inventive method.

[0067] The dose of the inventive gene transfer vector administered to a mammal, particularly a human, in the context of the invention will vary with the particular gene transfer vector, the composition containing the gene transfer vector, the method of administration, and the particular site being treated. The dose should be sufficient to effect a desirable response, preferably a humoral immune response against *B. anthracis* infection, within a desirable time frame. When the inventive gene transfer vector is an adenoviral vector, typical doses will contain at least about 1×10^5 particle units (pu) of the adenoviral vector (e.g., at least about 1×10^6 pu), preferably at least about 1×10^7 pu (e.g., at least about 1×10^8 pu). Higher doses also can be used, such as doses of at least about 1×10^9 pu

(e.g., at least about 1×10^{10} pu), or even at least about 1×10^{11} pu (e.g., at least about 1×10^{12} pu), or even higher, such as at least about 1×10^{13} pu (e.g., at least about 1×10^{14} pu). Generally, dosages will be about 1×10^5 - 1×10^{14} pu (e.g., about 1×10^7 - 1×10^{13} pu), preferably 1×10^8 - 1×10^{12} pu (e.g., about 1×10^9 - 1×10^{11} pu).

[0068] With respect to the number of administrations of the inventive gene transfer vector, the most preferred dosing schedule involves a single administration of a dose of the gene transfer vector to the host. However, if a single administration of the inventive gene transfer vector does not elicit a sufficient humoral immune response against *B. anthracis* infection, a second dose can be administered to the host. The inventive method preferably comprises no more than two administrations of the inventive gene transfer vector to any one host. Where the gene transfer vector is an adenoviral vector and a second administration of the adenoviral vector is required, the first and second administrations can employ any combination of adenovirus strains. For example, the first administration can employ a human adenovirus, while the second administration can employ a chimpanzee adenovirus, and vice versa. In that human adenovirus serotype 5 and chimpanzee adenovirus strain C68 are preferred for use in connection with the inventive method, possible combinations of first and second administrations include: (i) Ad5 followed by Ad5, (ii) Ad5 followed by AdC68, (iii) AdC68 followed by AdC68, and (iv) AdC68 followed by Ad5.

Other Considerations

[0069] In addition to utilizing heterologous signal peptides, the humoral immune response to anthrax infection also can be enhanced by potentiating the interaction of anthrax exotoxins expressed by the gene transfer vector with antigen presenting cells (e.g., dendritic cells). Thus, in particular embodiments of the invention, the nucleic acid sequence can encode a genetic adjuvant to enhance the participation of dendritic cells in the humoral immune response. A genetic adjuvant desirably enhances dendritic cell (DC) participation by either activating dendritic cells upon introduction or infection of the inventive gene transfer vector, or by recruiting more dendritic cells to the site of gene transfer vector introduction or infection. The nucleic acid sequence may encode any suitable genetic adjuvant known in the art that activates or recruits dendritic cells, such as, for example, a cytokine. When the nucleic acid sequence encodes a genetic adjuvant that activates dendritic cells, preferred genetic adjuvants include CD40 ligand (CD40L), p65RHD, which is the N-terminal fragment of the p65, cRel NF6B gene, and intercellular adhesion molecule (ICAM). When the nucleic acid sequence encodes a genetic adjuvant that recruits dendritic cells to the site of infection, preferred genetic adjuvants include MIP3- α , SDF1, and MDC. The nucleic acid sequence can encode one or more genetic adjuvants in any combination. In this respect, the nucleic acid sequence can encode (i) one or more DC-activating

adjuvant(s), (ii) one or more DC- recruiting adjuvant(s), or (iii) one or more DC-activating adjuvant(s) and one or more DC-recruiting adjuvant(s).

[0070] The inventive method can be performed in combination with other methods for the prophylaxis or treatment of *B. anthracis* infection. In one embodiment, the inventive gene transfer vector can be administered in conjunction with an existing anthrax vaccine, such as the U.S. anthrax vaccine available from Bioport Corporation, and the rPA/Alhydrogel vaccine described elsewhere herein. In addition, the inventive gene transfer vector can be administered before, after, or concurrently with antibiotics approved for use in managing *B. anthracis* infection. Such antibiotics include ciprofloxacin, doxycycline, and penicillin G. Alternatively, levofloxacin or ofloxacin may be administered instead of ciprofloxacin (see, e.g., Cieslak et al., *Emerg. Infect. Dis.*, 5, 552-555 (1999), and Bell et al., *Emerg. Infect. Dis.*, 8, 222-225 (2002)). In addition, other therapeutic methods suggested in the art to exhibit anti-exotoxin properties can be used in conjunction with the inventive method. Such therapies include the use of clindamycin, steroids (particularly for the treatment of cutaneous forms of anthrax), angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers and tumor necrosis factor inhibitors (see, e.g., Bell et al., *supra*).

[0071] Because *B. anthracis* is an infectious agent which causes serious, and sometimes lethal, disease as a result of inhalation, all manipulations involving any component of the *B. anthracis* pathogen (e.g., the *B. anthracis* genome, spores, proteins, etc.) must be performed in accordance with Biosafety Level 3 (BSL 3) regulations as set forth by the Centers For Disease Control and Prevention (CDC) (<http://www.cdc.gov/>).

[0072] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0073] This example demonstrates the generation of a gene transfer vector comprising a nucleic acid sequence encoding an immunogenic portion of protective antigen of *Bacillus anthracis* and a heterologous sorting signal, wherein the nucleic acid sequence comprises codons expressed more frequently in humans than in *Bacillus anthracis*.

[0074] Using routine molecular biology techniques, plasmids were constructed to express a nucleic acid sequence encoding *B. anthracis* protective antigen comprising human-preferred codons (hPA), the sequence of which is set forth in SEQ ID NO:1. Plasmid sec-hPA contains a nucleic acid sequence encoding the cleavable LAMP-1 signal peptide and the nucleic acid sequence encoding hPA, which was designed to direct the secretion of hPA. Plasmid sec-hPA-LAMP1 is identical to the sec-hPA plasmid, but was

further engineered to express the LAMP-1 sorting signal (described in, e.g., Wu et al., *supra*) at the C-terminus of hPA. The sec-hPA-LAMP1 plasmid was designed to target hPA to the endosome/lysosome compartment. Both constructs were cloned into expression plasmids and operably linked to the cytomegalovirus immediate-early (CMV IE) promoter-enhancer. In addition, the sec-hPA-LAMP-1 expression construct was inserted into the E1 region of a replication-deficient adenoviral vector of serotype 5 (AdsechPA), using techniques known in the art and described herein. An E1-deficient adenoviral vector without any transgene (AdNull) was used as a control. The resulting adenoviral vector was propagated in 293 cells (see Graham et al., *supra*), which complement for E1 deficiencies.

[0075] To test for expression of hPA and for induction of an anti-hPA immune response, the plasmids and adenoviral vectors were administered to C57Bl/6 (B6) mice by intramuscular injection. Specifically, the mice received 50 µg plasmid/100 µl PBS, or 10⁹ particle units (pu) of adenoviral vector. At two weeks post injection, blood was drawn and assessed by ELISA for the presence of anti-PA antibodies. Recombinant hPA was recognized by serum from mice immunized by the sec-hPA-LAMP-1 plasmid but not by serum from mice injected with the sec-hPA plasmid. In addition, the AdsechPA vector produced a higher anti-PA antibody titer in immunized mice than did the sec-hPA-LAMP1 plasmid vector.

[0076] The results of this example demonstrate the production of a gene transfer vector comprising a nucleic acid encoding a humanized immunogenic portion of *Bacillus anthracis* protective antigen and a heterologous sorting signal.

EXAMPLE 2

[0077] This example demonstrates a method of producing an immune response against *Bacillus anthracis* in a host by administering the inventive gene transfer vector to the host.

[0078] The immune response to a single intramuscular administration of AdsechPA (see Example 1) in BALB/c mice was compared with the recombinant PA protein/Alhydrogel vaccine (rPA/Alhydrogel) described herein, which was generated as described in Little and Knudson, *Infect. Immun.*, 52, 509-512 (1986). In this respect, a dose of 3x10⁹ pu of AdsechPA and a 25 µg dose of the rPA/Alhydrogel vaccine were administered separately to BALB/c mice. Saline injections and Adnull were administered as controls. Anti-PA antibody production was assessed using ELISA.

[0079] A time course study of anti-PA IgG titer demonstrated that, at two weeks post administration, the IgG levels produced by each vaccine diverged, with the AdsechPA vaccine yielding a 10-fold higher titer. Assessment of IgG subclasses showed that, at four weeks post administration, the AdsechPA and rPA/Alhydrogel vaccines induced similar titers of IgG1, but the AdsechPA vaccine yielded greater than 200-fold higher IgG2a,

IgG2b, and IgG3 titers than the rPA/Alhydrogel vaccine. The time course study also demonstrated that, over time, the 10^9 dose of AdsechPA yielded higher anti-lethal toxin (i.e., PA + LF) neutralizing antibody titers than the rPA/Alhydrogel vaccine. Indeed, at two weeks post administration, the AdsechPA vaccine elicited a neutralizing antibody titer of greater than 10^2 , while the neutralizing antibody titer elicited by the rPA/Alhydrogel vaccine was barely detectable

[0080] In addition, immunized mice were challenged with lethal toxin by intravenous injection of mixed rPA and recombinant LF. Neutralizing anti-PA or anti-LF antibodies produced from lethal toxin challenge were quantified by a macrophage protection assay (see, e.g., Friedlander, *J. Biol. Chem.*, 261, 7123-7126 (1986)). A single intramuscular administration of a 2.5 μ g dose of the rPA/Alhydrogel vaccine protected only 7.6% of the mice challenged with lethal toxin, while nearly all of the animals receiving a 3×10^9 pu dose of AdsechPA were protected. In addition, the same dose of AdsechPA was shown to offer partial protection to lethal toxin challenge as early as 11 days post administration, while the rPA/Alhydrogel vaccine offered no protection against lethal toxin challenge at the same time point.

[0081] The results of the example demonstrate the production of a rapid and efficient anti-PA immune response elicited by the inventive gene transfer vector, as well as protection against subsequent anthrax infection.

EXAMPLE 3

[0082] This example demonstrates a method of producing an anti-PA immune response using a human or chimpanzee adenoviral vector comprising a nucleic acid encoding a humanized immunogenic portion of PA and a heterologous sorting signal.

[0083] Using techniques known in the art and described herein, the sec-hPA-LAMP1 expression construct of Example 1 is inserted into the E1 region of a replication-deficient chimpanzee adenoviral vector of strain C68 (AdC68sec-hPA-LAMP1). Mice from three different laboratory strains, C57Bl/6, A/J, and ICR, are administered the Ad 5-based adenoviral vector of Example 1 (AdsechPA), or AdC68sec-hPA-LAMP1 via any one of the following routes: intravenous injection, intramuscular injection, oral administration, nasal administration, and injection into the foot pad. As a control, the response produced by each adenoviral vector is compared to the response produced by recombinant PA in alum.

[0084] Immunized mice are assessed for anti-PA humoral responses by bleeding from the tail vein at multiple time points, including pre-vaccination, 1, 2, 4, 8, 16, and 26 weeks post infection (p.i.), and by quantifying anti-PA antibodies using ELISA. In addition to humoral responses, cell-mediated (i.e. T cell) responses are assessed at days 7 and 14 p.i. using the ELISPOT assay described above. At 4 and 16 weeks post infection, mice are

challenged with PA+LF toxin or the Sterne strain of *B. anthracis*. Candidate gene transfer vectors that induce the strongest protective response are identified and reassessed at time points closer to the time of vector administration (i.e., days 1, 2, 3, 5, and 7 p.i.).

[0085] The efficacy of candidate adenoviral gene transfer vectors in the presence of preexisting anti-Ad5 neutralizing antibodies is assessed in mice pre-immunized with 1×10^9 pu of wild-type Ad5 four weeks prior to administration of candidate gene transfer vectors. The presence of anti-Ad5 neutralizing antibodies is determined as the inhibition of Ad5-induced cytopathic effect on A549 cells. Once optimal doses are determined as a result of the above described assays, the immune responses elicited by the “best” candidate Ad5 and/or AdC68 vaccines are assessed in naive and Ad5-immunized mice. The candidate vectors are administered in two doses administered four weeks apart, and in all possible combinations (i.e., Ad5 followed by Ad5, AdC68 followed by AdC68, Ad5 followed by AdC68, and AdC68 followed by Ad5).

[0086] This example demonstrates a method of producing an anti-PA immune response using a human or chimpanzee adenoviral vector comprising a nucleic acid encoding a humanized immunogenic portion of *Bacillus anthracis* protective antigen and a heterologous sorting signal.

EXAMPLE 4

[0087] This example demonstrates a method of producing an immune response against *Bacillus anthracis* in a host, which comprises administering to dendritic cells of the host a gene transfer vector comprising a nucleic acid sequence as described in Example 1.

[0088] Using methods described in, for example, U.S. Patents 5,770,442 and 5,965,541, the penton base integrin-binding RGD domain and (separately) a seven-residue polylysine (pK7) sequence are engineered into the fiber knob domain of a replication-deficient Ad 5 adenoviral vector and an AdC68 adenoviral vector, each encoding a reporter gene (e.g., luciferase). Such modifications to the fiber protein of Ad 5, have been demonstrated to preferentially direct adenoviral infection to dendritic cells as described elsewhere herein.

[0089] Dendritic cell infection by the fiber-modified AdC68 vector is assessed by infecting human monocyte-derived DC with 100 plaque forming units (pfu)/cell of the fiber-modified AdC68 vector and incubating for 48 hours. Luciferase expression is measured using standard techniques and is indicative of DC gene transfer by the fiber-modified AdC68 vector.

[0090] The fiber-modified Ad5 and AdC68 vectors most effective at DC gene transfer are engineered to contain the sec-hPA-LAMP1 expression construct (see Examples 1-3) and are tested using the mouse models described in Example 3. In this respect, both the humoral anti-PA immune response and the anti-lethal toxin protective immune response

elicited by hPA expression are assessed and compared to the immune responses generated by identical Ad5 and AC68 gene transfer vectors with wild-type capsid proteins.

[0091] This example demonstrates a method of producing an immune response against *Bacillus anthracis* in a host by administering the inventive gene transfer vector to dendritic cells of the host

[0092] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0093] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0094] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.